



## Food resources influencing the asexual reproductive cycle of coronate Scyphozoa

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**Abstract:** The study of the strobilation process, a feature unique in the class Scyphozoa, is an issue that helps understanding the patterns of asexual reproduction in sessile invertebrates. Many inducers of asexual reproduction are known for scyphozoans. However, the influence of food resources on the strobilation of Coronate Scyphozoa has never been tested. We observed strobilation of a large number of polyps of *Nausithoe aurea*, from a wide sampling area along the South Atlantic coast of Brazil, through the administration of controlled number of hatched nauplii of *Artemia franciscana* under a previous tested starvation and feeding protocol. The number of strobilations between and within groups varied and the fate and shape of strobilation deviated from the biology reported in the original description. Artificial seawater was used to reduce the influence of dissolved organic matter as likely important alternative nourishment.

**Resumé :** Influence de la ressource alimentaire sur le cycle de reproduction asexuée des Scyphozoa Coronata. L'étude de la strobilation dans la classe des Scyphozoa est importante pour comprendre les modes de reproduction asexuée chez les invertébrés sessiles inférieurs. De nombreux inducteurs de la reproduction asexuée des Scyphozoa sont connus. Toutefois, l'influence des ressources alimentaires sur la strobilation des Scyphozoa Coronata n'a jamais été testée. Nous avons observé la strobilation d'un grand nombre de polypes de *Nausithoe aurea*, provenant d'une vaste zone d'échantillonnage le long de la côte du Brésil en Atlantique Sud, en contrôlant l'apport du nombre de nauplii d'*Artemia franciscana*, dans le cadre d'un protocole déjà testé précédemment d'alimentation et de jeûne. Le nombre de strobilations entre et au sein des groupes a varié et le devenir et la forme de la strobilation a différé des données biologiques issues de la description originale. L'eau de mer artificielle a été utilisée pour réduire l'influence de la matière organique dissoute comme importante source de nutriments.

**Keywords:** Strobilation • Dissolved Organic Matter • Food induction • Polyps

## Introduction

The class Scyphozoa is characterized by strobilation, a term used to describe the production of discs by transverse fissions of the body column, metamorphosing the scyphistoma (or polyp) structure into a young medusa, or ephyra (Arai, 1997). Members of the order Coronatae have a periderm tube encircling the soft body, and strobilate, producing an operculum of periderm or of simple tissue in the aperture of the tube (Werner, 1970). For example, *Atorella vanhoeffeni* Bigelow, 1909, strobilates with a tissue cover and produces more than 200 minute ephyrae (Werner, 1967); *Nausithoe maculata* Jarms, 1990, *N. marginata* Kölliker, 1853 and *N. weneri* Jarms, 1990, strobilate with a peridermic operculum and produce more than a 100 ephyrae (Jarms, 1990).

As a general pattern during strobilation for scyphozoans (The basal part of the strobilating polyp does not lose inter-radial septa with muscle tube (Eggers & Jarms, 2007)) the upper part of the remainder of the scyphistoma column begins to restructure, by developing mesenteries, and later, the oral disc (Spangenberg, 1968; Arai, 1997). In restructuring, the scyphistoma probably makes use of undifferentiated tissues to create new tentacles and mesenteries (Lesh-Laurie & Corriel, 1973).

Silveira & Morandini (1998) described the asexual segmentation for the colonial species *Linuche unguiculata* (Swartz, 1788), also considered a mechanism to rejuvenate the polyp tissues. Silveira et al. (2003) observed segmentation in *Nausithoe aurea* Silveira & Morandini, 1997, and suggested that production of the peridermic operculum is controlled by the intrinsic regulation to strobilate, first by the surplus of food energy and, second segmentation, as variation upon strobilation. The extreme of regeneration is observed in *Aurelia*, in which isolated polyp tentacles regenerate into an entire polyp (Lesh-Laurie & Corriel, 1973; Lesh-Laurie et al., 1991).

Temperature changes and light regime have been suggested to induce strobilation in *Chrysaora quinquecirrha* (Desor, 1848) (Thiel, 1962; Loeb, 1972 & 1973; Purcell et al., 1999). Chiba (1969) reported the influence of ecological factors on strobilation of *Aurelia aurita*. Chen & Ding (1983), Chen et al. (1984 & 1985) showed a sequence of publications about the ecological influences of nutritional condition, light and temperature on the strobilation of *Rhopilema esculenta*. However, these studies only report the quality of strobilations during these experiments and observations.

A number of benthic marine invertebrates have been studied with respect to focusing their nutrition sources, with emphasis on the mechanism of alternative nourish-

ment by Dissolved Organic Matter (DOM) (Southward & Southward, 1972). DOM can affect the trophic chains in different ways in the marine environment: first, it can be used directly as a food resource; second, as a seasonal marker for primary producers (Ganachaud & Wunsch, 2002).

Following the work on survivorship of *Nausithoe aurea* (Silveira & Morandini, 1997) in nature and in the laboratory (Silveira et al., 2003), the focus of the present study was to establish a methodology to demonstrate the importance of food resources for the asexual reproduction processes and related phenomena. The possible significance of DOM in the life cycle of the species is also discussed.

## Material and Methods

The species *Nausithoe aurea* described by Silveira & Morandini (1997), was reared in the laboratory (see details in Jarms et al., 2002) from solitary scyphistomae collected in shallow waters of the São Sebastião Channel (São Paulo state, southeastern Brazil). The internal cusps are 16 in number, and they are arranged in a regular series containing four large, four medium, and eight small ones. The typical scyphistomae have 16 to 31 filiform tentacles with conspicuous white spots between their bases (Silveira & Morandini, 1997).

Polyps from different localities along the Brazilian coast (covering the distributional area of the species; see Morandini et al., 2005) were used in the experiments to assess variability among the scyphistomae. The polyps were sampled from: Bahia State (BA), Taipús (13°56'S - 38°55'W); Espírito Santo State (ES), Aracruz (19°50'S - 40°03'W); Rio de Janeiro State (RJ), Angra do Reis, Ilha Grande (23°01'S - 44°19'W); São Paulo State (SP), São Sebastião Channel (23°51'S - 45°25'W), the type locality; and Santa Catarina State (SC), Ilha do Arvoredo (27°17'S - 48°22'W).

The polyps were divided into four experimental groups (A, B, C, D) and were observed in different periods of time; first groups A and B (08-11/2004), and later C and D (01-05/2005). Each polyp was maintained individually for 105 days in Polystyrene dishes (Corning, No.6 series 430165, 3.5 cm diameter) filled with 10 ml of artificial seawater (ASW) (Tropic Marin®) salinity 35 (refractometer Atago, model S/Mill-e, error probability of 0.5). The water of each dish was changed daily. Groups A, B and C were composed of 30 polyps from the São Sebastião Channel (SP) (three replicas). Group D was composed of polyps from different locations: BA (10), ES (10), RJ (5) and SC (5) (see abbreviations above). All groups were maintained in controlled temperature chambers (22°C ± 0.5), in complete darkness (adapted from Jarms et al., 2002).

The experiments consisted of feeding-starvation periods of, respectively, 21 and 14 days. This protocol was previously established and tested (Stampar & Silveira, 2006). Prior to the start of the observations, all polyps were starved for 2 weeks in ASW to provide a “gastric cleaning” and level the nutritional energy of the animals.

During the feeding periods, we offered 5 nauplii (24 hours after hatching) of *Artemia franciscana* Kellog, 1906 (MARAMAR®) daily. This quantity corresponds to 0.0275 calories available per day (Maciolek, 1962). On the following day, each dish was examined, its water was changed, and the remnants of nauplii were counted to estimate the daily number of nauplii ingested per polyp in each group (A, B, C and D).

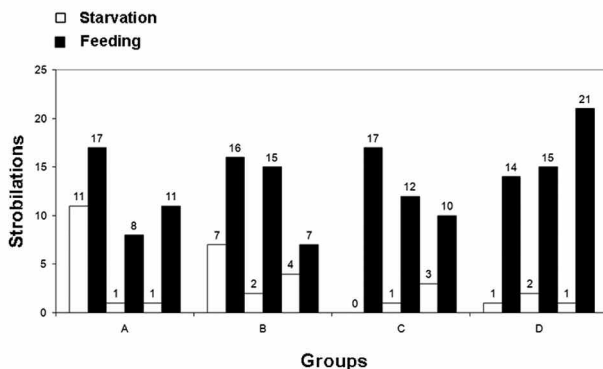
To avoid the addition of organic matter in the artificial seawater, we used ultrapure and expected particle (organic and inorganic) – free water filtered with Millipore® Elix 5 (Analytical-Grade Water).

The results analysis was made using the softwares Minitab 14 and SPSS 14.0. In the statistical analysis we first built a null hypothesis ( $H_0$ ) and performed a Z-test to analyze the differences between the strobilations sum during the periods of starvation and feeding. After we set up a hypothesis test to verify the distribution and then plotted that in relation to critical region (rejection region).

## Results

### Food inducing strobilation

From our experiments (starvation and feeding), we observed that the ingestion of food induced strobilation. In

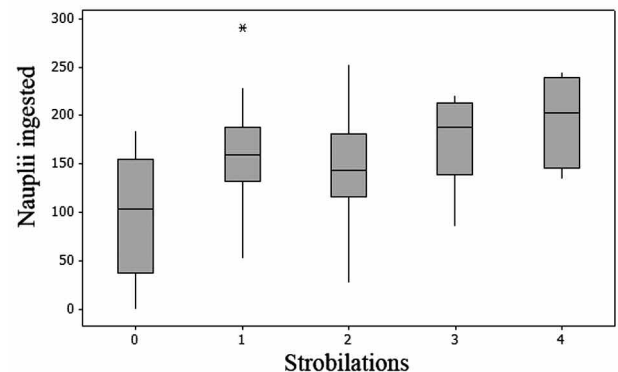


**Figure 1.** *Nausithoe aurea*. Number of strobilations in each experimental group (A, B, C, and D), for starvation and feeding periods during the 105 days of the experiment.

**Figure 1.** *Nausithoe aurea*. Nombre de strobilations de la *N. aurea* dans chaque groupe expérimental (A, B, C et D), au cours des périodes de jeûne et d'alimentation pendant les 105 jours d'expérimentation.

all groups we observed more strobilations during the feeding periods than during the starvation periods (Fig. 1).

The variation of ingested nauplii by the observed number of strobilations is shown in Fig. 2. Induction of strobilation was almost similar in all groups. However, the number of strobilations between and within each group varied, as shown in Fig. 1.



**Figure 2.** *Nausithoe aurea*. Relationship between the number of ingested nauplii and the number of strobilations during the experiments.

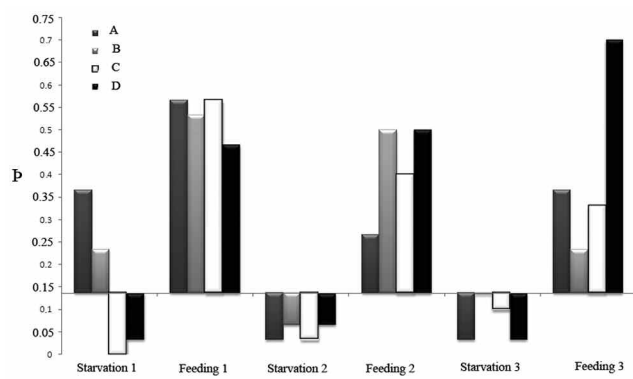
**Figure 2.** *Nausithoe aurea*. Relation entre le nombre de nauplii ingérées et le nombre de strobilations au cours des expérimentations.

We first tested the null hypothesis ( $H_0$ ) that the mean number of strobilations in the starvation periods did not show significant differences from the mean number in the feeding periods. We set the significance level ( $p$ ) at 0.05, resulting in a value of  $Z_{0.05} = 1.96$ . Comparing the mean number of strobilations in the starvation and feeding periods, we obtained  $Z_{\text{Experimental}} = 9.08 > Z_{0.05} = 1.96$ ; thus, we rejected the null hypothesis ( $H_0$ ), that there was no statistical difference between the mean number of strobilations during the starvation and feeding periods.

We then established a hypothesis test for each experimental group (A, B, C, and D) to verify the data distribution in each group. We determined the general critical region ( $C$ ) (to represent the whole experiment without emphasizing any group). This was determined as:  $C = P \leq 0.1362$ . After that, we determined the values of individual  $P$  for each period and group (Fig. 3); note that the X-axis crosses the Y-axis at the level of the general critical region ( $C = 0.1362$ ).

### Fate and shape of strobilation disks and chain

During the course of the experiments, some scyphistomae



**Figure 3.** *Nausithoe aurea*. Values of  $\bar{P}$  for each experimental group and period (starvation and feeding).

**Figure 3.** *Nausithoe aurea*. Valeurs de  $\bar{P}$  pour chaque groupe expérimental et période (jeûne et alimentation).

showed differences from the initial strobilation observations by Silveira & Morandini (1997). Some disks were not released by some polyps. There was no disk liberation in 82 out of 197 strobilations. In these strobilations we also observed a number of modifications from the 'expected pattern of strobilation' for the species, following 11 years of experience in rearing *Nausithoe aurea* in the laboratory (since 1996). We describe these differences below (see also Fig. 4), which can be observed by the modified aspect of early strobilation.

In the expected pattern, the regularly shaped disks become ephyrae that are later released or that transform into planuloids (see description in Silveira & Morandini, 1997). In the altered pattern, irregularly shaped disks produce deformed ephyrae that are not released at all or are released as planuloids. On 82 occasions, the strobila developed thin, irregular disks (Fig. 4).

## Discussion

### Food inducing strobilation

The differences between the starvation and feeding periods are clearly seen in Fig. 3. On one hand, all  $\bar{P}$  values of the feeding periods were above of the critical region; and almost all  $\bar{P}$  values of the starvation periods were below the critical region. On the other hand, the two  $\bar{P}$  values of the first starvation periods referring to groups A and B were above the critical region. We explain the strobilations occurring in these groups by the induction produced by the sterile polystyrene dishes. New polystyrene dishes are a known inducer of strobilation in other scyphozoans (Herrmann et al., 2003; Berking et al., 2005) and also in the species studied (Stampar et al., 2007); note that the periods



**Figure 4.** *Nausithoe aurea*. Development of strobilum in *N. aurea*, expected patterns (I, III) and altered patterns (II, IV). Note: I and II show the onset of strobilation, and III and IV show the beginning of disk formation.

**Figure 4.** *Nausithoe aurea*. Développement du strobile, schémas attendus (I, III) et modifiés (II, IV). Note: I et II montrent l'apparition de la strobilation, III et IV montrent le début de la formation du disque.

and groups in which we used sterile dishes were A and B during the first starvation.

Based solely on the comparisons of the number of strobilations during the starvation and feeding periods, we can state that strobilation was induced by food availability in *Nausithoe aurea*. This result was expected based on some previous studies (Calder, 1974; Purcell et al., 1999). However, this work shows the first evidence with all controlled factors. In Calder (1974) the effects of the water condition and the influence of food stocks were not controlled. In Purcell et al. (1999) the influence of Dissolved Organic Matter was reduced by micro-filters.



However, the valuable data about the feeding of each polyp was not measured.

The statistical analysis showed very strong defined strobilation groups. Nevertheless, to corroborate the probability of the correct decision in relation to the difference between starvation and feeding periods, we made another test to evaluate the error probability, analysing the value of  $P_{\text{general}} = 0.2705$ . The test of error probability gave a result of 97.83% for the right decision, which is a safe level for this kind of experiment (see Callegari-Jacques, 2003; Wayne, 2004).

#### *Fate and shape of strobilation disks and chain*

The data obtained in this study, rearing of the scyphistomae in ASW, were compared with specimens reared in natural seawater (Silveira & Morandini, 1997; Jarms et al., 2002). Rearing small invertebrates in small containers is more convenient to work with natural seawater, especially in coastal regions. However, seawater may vary widely from season to season. The use of ASW controls the water quality, allowing the repetition of the work in the future (Shick, 1975; Berges et al., 2001).

Initially, the seawater was regarded only as a background or "culture medium" to devise the experiments. Conversely, at the beginning of the 20<sup>th</sup> Century a number of workers began to study the relationships of the DOM with the system productivity and its role in the environment (see more in Duursma, 1961).

The effect of DOM on scyphozoans was studied by Shick (1975), who determined its influence on the development of *Aurelia aurita* (Linnaeus, 1758). The quality of the seawater in different periods was important for the reproduction of *A. aurita* in nature.

In the present study, the influence of DOM was much minimized by the methods, as shown by the different shapes of the strobila and note worthy the production of thin irregular disks (Stampar & Silveira, 2006). The strobilus probably uses DOM as a food resource during the strobilation period (when they cannot feed normally) by simple diffusion through its body.

Finally, the factors that cause the alterations in strobila resulted in reduction of releases (ephyrae or planuloids). These factors were probably created by rearing the animals in artificial seawater. The idea of the use of DOM by scyphistomae is suggested.

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